Adenoviral Infection Presenting as an Isolated Central Nervous System Disease without Detectable Viremia in Two Children after Stem Cell Transplantation[▽]

Pierre Frange,[1]* Régis Peffault de Latour,[2] Cécile Arnaud,[3] Nathalie Boddaert,[4] Mehdi Oualha,[5] Véronique Avettand-Fenoel,[6] Françoise Bernaudin,[3] Claire Aguilar,[7] Christine Barnerias,[8] Marianne Leruez-Ville,[6] Fabien Touzot,[1] Olivier Lortholary,[7] Alain Fischer,[1] and Stéphane Blanche[1]

Unité d'Immunologie, Hématologie et Rhumatologie pédiatriques, Assistance Publique-Hôpitaux de Paris (APHP), Hôpital Necker-Enfants malades, Paris, France[1]; Service d'Hématologie-Greffe, APHP, Hôpital Saint Louis, Paris, France[2]; Service de Pédiatrie, Centre de Référence de la Drépanocytose, Centre hospitalier intercommunal, Créteil, France[3]; Service de Radiologie pédiatrique, APHP, Hôpital Necker-Enfants malades, Paris, France[4]; Service de Réanimation polyvalente pédiatrique, APHP, Hôpital Necker-Enfants malades, Paris, France[5]; Laboratoire de Virologie, APHP, Hôpital Necker-Enfants malades, Paris, France[6]; Université Paris Descartes, Service de Maladies Infectieuses et Tropicales, APHP, Hôpital Necker-Enfants malades, Paris, France[7]; and Service de Neurologie pédiatrique, APHP, Hôpital Necker-Enfants malades, Paris, France[8]

We report two cases of adenoviral meningoencephalitis in children following allogeneic stem cell transplantation. These cases showed four similarities: isolated neurological involvement, infiltrating hyperintensities next to the third ventricle on the cerebral magnetic resonance image, the absence of concomitant detectable adenoviral viremia, and a severe clinical outcome.

CASE REPORTS

The first child suffered from severe sickle cell anemia. She experienced pneumococcal septic shock when she was 13 months old with bilateral ischemic frontal and occipital lesions revealed by cerebral magnetic resonance imaging (MRI). At the age of 14 years, she underwent hematopoietic stem cell transplantation (HSCT) from a matched sibling donor after a conditioning regimen including busulfan, cyclophosphamide and antithymocyte globulins. Prophylaxis against graft-versus-host disease (GVHD) initially consisted of methotrexate and cyclosporine and was switched to steroids on day 6 after HSCT. A few hours after the graft infusion, the patient complained of transient acute headache without other neurological symptoms. Examination of cerebrospinal fluid (CSF) revealed an isolated high protein concentration (0.53 g/liter). This result was attributed to a traumatic tap, as cytological examination exhibited a high red blood cell count and a normal white blood cell count (Table 1). The cerebral MRI finding was normal, except for the ischemic lesions due to the known vasculopathy. Hematological recovery was attained on day 15 with 100% donor chimerism. The immunosuppressive regimen was progressively switched from steroids to mycophenolate mofetil starting on day 45. At the same time, the child presented a cytomegalovirus (CMV) infection (plasma viral load of 5.9 $\log_{10}$ DNA copies/ml) with fever and asthenia. Foscarnet therapy for 2 weeks and then ganciclovir were administered. PCR

testing for plasma CMV was negative on day 103. On day 77, grade II to III cutaneous and digestive GVHD required an increased dose of steroids, with rapid remission of the symptoms. Steroids were stopped on day 118. On day 121, the patient presented headache, fever, shivers, and tremors. All microbiological tests of blood samples were negative, including PCR assays for CMV and adenovirus. On day 123, the child was delirious and confused and CSF examination revealed an isolated high protein concentration (0.57 g/liter), with microbiological analyses all scoring negative. The investigations of successive CSF samples are summarized in Table 1. Concomitant cerebral MRI showed a hyperintensity infiltrating the fornix (Fig. 1A, arrow). Because microbiological tests were all negative, the diagnosis of possible immunological vasculitis was considered and steroid therapy was reintroduced. After a transient improvement of fever, asthenia, and tremors, neurological symptoms gradually worsened on day 148. The child developed a dementia syndrome and a severe pyramidal and extrapyramidal syndrome. On day 176, she was admitted to the intensive care unit because of autonomic dysfunction complicated by acute respiratory failure (aspiration pneumonia) and septic shock. Investigations of the CSF revealed a high protein concentration (1.36 g/liter) and the presence of adenovirus species D as assessed by PCR (4.5 $\log_{10}$ DNA copies/ml). No other bacterial, viral, or fungal pathogen was identified in CSF specimens. Concomitant PCR tests for adenovirus in stool samples were positive, but the blood remained negative. Retrospectively, we assumed that the elevated protein concentration in the previous CSF sample (taken on day 123) may have been due to viral meningoencephalitis, with an adenovirus DNA load below the detection limit of the PCR test used. On day 189, cerebral MRI revealed hyperintensities around the

TABLE 1. Results of investigations of CSF from two children with adenoviral meningoencephalitis following bone marrow transplantation

Patient and time of CSF sampling (days after transplantation)	No. of white blood cells/μl	Cytospin morphological analysis	No. of red blood cells/μl	Glucose concn (mmol/liter)	Protein concn (g/liter)	Gram stain	Bacteriological culture	Adenovirus PCR (log$_{10}$ no. of DNA copies/ml)
No. of days 1								
6	2	NP[a]	200	3	0.53	Negative	Sterile	NP
123	3	NP	1	2.8	0.57	Negative	Sterile	Negative
176	4	NP	1	4	1.36	Negative	Sterile	4.5
187	1	NP	54	NP	NP	Negative	Sterile	5.1
No. of days 2								
86	7	Majority of lymphocytes	0	2.6	0.36	Negative	Sterile	3.1
147	16	Majority of lymphocytes	0	2.4	0.29	Negative	Sterile	Negative

[a] NP, not performed.

third and fourth ventricles. These hyperintensities infiltrated the thalami, the chiasma and optic structures, and the middle temporal lobes within the amygdala and hippocampi and extended inferiorly near the fourth ventricle into the brain stem, suggesting rhombencephalitis (Fig. 1D to F). A treatment involving mechanical ventilation, catecholamine infusions, antibiotics, high-dose intravenous (i.v.) immunoglobulins, and 5 mg/kg weekly cidofovir improved the respiratory and hemodynamic status. However, the neurological status remained poor (Glasgow coma scale score of 3 to 4), with a late development of diabetes insipidus and progressive brain stem impairment. PCR tests for adenovirus in the blood remained negative, but despite the antiviral treatment, the adenovirus DNA load in the CSF increased (to 5.1 log$_{10}$ DNA copies/ml on day 187). The child died on day 197, 21 days after her admission to the intensive care unit.

The second child had primary immunodeficiency due to a major histocompatibility complex class II expression deficiency caused by a homozygous mutation in the *RFXANK* gene. During her first years of life, she repeatedly presented with upper and lower respiratory tract infections with subsequent bronchial dilatation. Systematic screening of her stool with real-time PCR tests for enterovirus and adenovirus at the age of 9 years revealed latent enteroviral and adenoviral C digestive infections, without clinical symptoms or detectable viremia. Two months later, she underwent HSCT from a matched sibling donor after a conditioning regimen including busulfan and cyclophosphamide. Prophylaxis against GVHD consisted of cyclosporine. Viral prophylaxis included i.v. acyclovir and high-dose immunoglobulin infusions because of the latent enteroviral infection. Hematological recovery was achieved on day 41 after transplantation, with 100% donor chimerism. On day 31, she presented with grade II to III cutaneous and digestive GVHD. Treatment with steroids and mycophenolate mofetil was followed by complete remission of the symptoms. Three weeks later, she presented with persistent fever with pyramidal signs and severe reduction of bilateral visual acuity contrasting with previous normal routine ophthalmologic examinations. The CSF analysis showed a limited pleocytosis (7 nucleated cells/μl, mainly lymphocytes) but normal glucose and protein levels. Microbiological analyses of the CSF included an isolated positive PCR result for adenovirus species C (3.1 log$_{10}$ DNA copies/ml; Table 1). No other bacterial, viral, or fungal pathogen was identified in CSF specimens. All microbiological tests of blood samples, including systematic weekly screening for adenovirus and enterovirus by PCR, were negative. Severe bilateral dysfunction of the optic nerves was detected by the pattern visual evoked potentials. The cerebral MRI showed infiltrating hyperintensity within the chiasmatic structures (Fig. 1B, arrow). Cidofovir infusions (5 mg/kg weekly) were administered for 2 months, with concomitant reduction of the immunosuppressive regimen. The fever disappeared, and PCR tests of the CSF were negative 2 weeks after the initiation of the treatment. Complete recovery of the pyramidal symptoms took several weeks. However, definitive severe reduction of bilateral visual acuity was noted. Two months after the beginning of the neurological symptoms, cerebral MRI revealed chiasmatic atrophy.

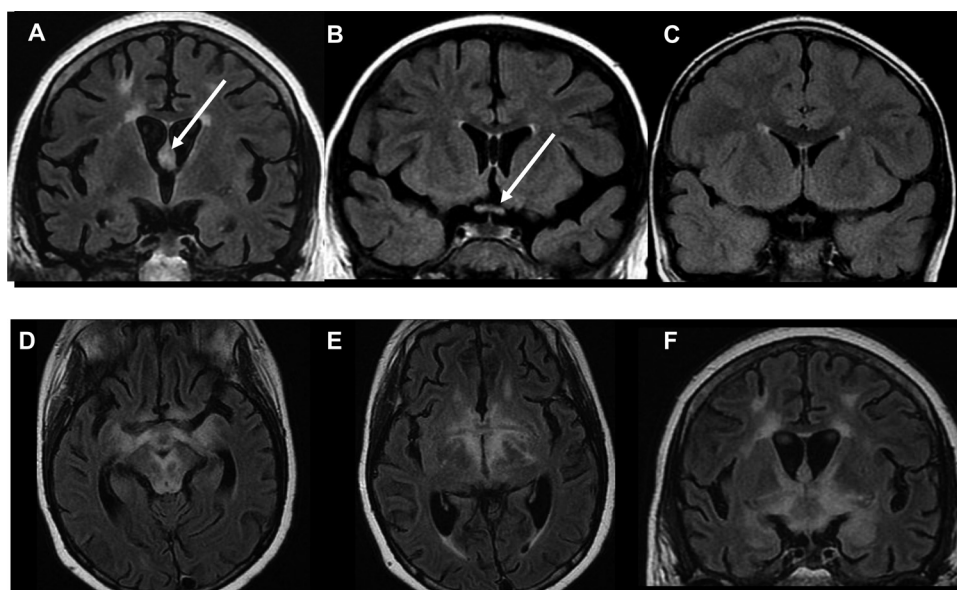Adenovirus is currently one of the most life-threatening viral

FIG. 1. MRI of the brain showing infiltrating hyperintensities next to the third ventricle in two children suffering from adenoviral meningo-encephalitis after HSCT. (A, B, C) Coronal FLAIR brain MRI images of the two affected girls (child 1, A; child 2, B) and a healthy child (C) for comparison. Note the infiltrating hyperintensity in the fornix on day 123 after HSCT in the first case (A, arrow) and the infiltrating hyperintensity in the chiasmatic structures on day 55 after HSCT in the second (B arrow). The fornix and chiasma are both close to the third ventricle. Periventricular hyperintensities in the first patient (A) were present before the adenoviral infection. (D, E, F) Axial (D, E) and coronal (F) FLAIR images of the first child showing the evolution after 2 months of infection (on day 189 after HSCT). The hyperintensities are apparent around the third ventricle and infiltrate the thalami, the chiasma and optic structures, and the middle temporal lobes within the amygdala and hippocampi and extend inferiorly near the fourth ventricle into the brain stem, suggesting rhombencephalitis.

pathogens in severely immunocompromised patients, especially children (1, 10, 12, 17). Five to 30% of HSCT recipients develop adenoviral infections during the posttransplantation period (1, 8, 17, 18, 22). Adenovirus infections are much more of a problem in pediatric patients undergoing HSCT than in adults because of the permanent circulation of the virus among children (22). Screening of patient blood for adenovirus DNA by quantitative PCR might help to avoid severe clinical complications, such as enteritis, pneumonitis, cystitis, and hepatitis (11). Weekly quantitative PCR to monitor the adenovirus DNA load is now common in many HSCT units (6, 12). In some cases, adenovirus can be detected significantly earlier at local sites, such as in stool samples or nasopharyngeal aspirates. Lion et al. demonstrated that the detection of adenovirus in stool samples by PCR tests is associated with disease and precedes adenoviral viremia by 11 days (13). Adenovirus DNA positivity of nasopharyngeal aspirates preceding HSCT has recently been suggested to be a very strong predictor of adenovirus viremia in children receiving HSCT from an unrelated donor (4). The second of our cases had chronic asymptomatic adenoviral carriage in stool samples before the HSCT. In the other case, adenovirus was isolated from stool samples and nasopharyngeal aspirates on the diagnosis of CNS infection. However, systematic screening of blood for adenoviral DNA by PCR was consistently negative in both cases during the post-HSCT period, including during adenoviral meningoencephalitis. It is, however, possible that any viremia was very transient and thus missed by weekly screening of plasma. To our knowledge, no such cases of invasive adenoviral infections without concomitant detectable viremia have previously been reported.

CNS involvement is rare in adenovirus-infected patients, and disseminated infections with multiorgan failure are more frequent in immunocompromised hosts (7, 10). Only one previous report described an adenovirus infection presenting primarily as a CNS disease (5).

Our patients developed an adenoviral infection after several weeks of increased immunosuppressive therapy secondary to acute GVHD. Both had profound T cell lymphopenia during the viral infection. These findings are consistent with previous studies of allogeneic HSCT populations, in which severe lymphopenia was a risk factor for disseminated viral infection with, in many cases, a fatal outcome (1, 9). However, two other reported risk factors of such infections were not present in our study: transplantation from an unrelated donor (versus sibling donor) and the use of a T cell-depleted graft.

Various features of adenoviral infection of the CNS have been described by MRI, including hydrocephalus with periventricular radiolucency and multiple parenchymal hypodensities, necrotic changes resembling herpes encephalitis (23), linear high signal intensity in the hippocampi on T1 and hyperintense signaling on FLAIR sequencing in both temporal lobes (5, 14), T2 signal abnormalities in the brain stem and cerebellum with mild patchy enhancement and mass effect (23). However, the substantial median infiltrating hyperintensity we observed has never been described previously, except in a 35-year-old HIV-positive woman with an unexplained basal forebrain mass and a postmortem diagnosis of adenoviral infection with electron microscopy examination (21).

One of our two cases involved species D and the other species C adenovirus. In the general population, most adenoviral infections are due to species B (serotypes 7 and 14) and C

(serotypes 1, 2, and 5) strains. Previous studies of patients with allogeneic stem cell transplants suggest that the most frequent adenovirus subgroup was C (2, 12, 17, 20). However, the strains isolated from adenoviral CNS infections are very diverse and include members of species A (serotype 31), B (serotypes 3 and 7), C (serotype 2), and D (serotypes 26 and 49 mixed with 31) (5, 14, 15, 19).

In both of the children described here, cidofovir treatment was introduced upon the diagnosis of adenoviral infection. This treatment was ineffective in the first case, with rapid clinical, radiological, and virological deterioration leading to death. In the second case, PCR tests for adenovirus in the CSF were negative after a few weeks of cidofovir therapy. However, the initial viral load in the CSF in the second case was moderate and much lower than that in the case with a fatal outcome. It is difficult to assess whether the virological improvement in the second case can be attributed to the antiviral treatment or to the simultaneous substantial reduction of the immunosuppressive regimen. Indeed, i.v. administered cidofovir does not seem to cross the blood-brain barrier efficiently (3). Further studies are needed to evaluate new antiviral drugs which can penetrate the blood-brain barrier so as to be effective in cases of isolated CNS infection. The recently developed, orally active, lipophilic form of cidofovir, CMX001, may be of value in such cases (16).

To conclude, adenoviral meningoencephalitis is a rare event which may be revealed by atypical clinical and radiological signs. Because of the severity of such infections, systematic screening by PCR for adenovirus in the CSF is required in cases of unexplained neurological symptoms in severely immunocompromised patients, such as children with allogeneic stem cell transplants, even if PCR tests of blood samples are negative.

REFERENCES

1. **Baldwin, A., et al.** 2000. Outcome and clinical course of 100 patients with adenovirus infection following bone marrow transplantation. Bone Marrow Transplant. **26:**1333–1338.
2. **Chakrabarti, S., et al.** 2002. Adenovirus infections following allogeneic stem cell transplantation: incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery. Blood **100:**1619–1627.
3. **Cundy, K. C., et al.** 1996. Pharmacokinetics, bioavailability, metabolism, and tissue distribution of cidofovir (HPMPC) and cyclic HPMPC in rats. Drug Metab. Dispos. **24:**745–752.
4. **de Pagter, A. P., et al.** 2009. Adenovirus DNA positivity in nasopharyngeal aspirate preceding hematopoietic stem cell transplantation: a very strong risk factor for adenovirus DNAemia in pediatric patients. Clin. Infect. Dis. **49:**1536–1539.
5. **Dubberke, E. R., et al.** 2006. Acute meningoencephalitis caused by adenovirus serotype 26. J. Neurovirol. **12:**235–240.
6. **Gustafson, I., et al.** 2008. Quantification of adenovirus DNA in unrelated donor hematopoietic stem cell transplant recipients. J. Clin. Virol. **43:**79–85.
7. **Hale, G. A., et al.** 1999. Adenovirus infection after pediatric bone marrow transplantation. Bone Marrow Transplant. **23:**277–282.
8. **Howard, D. S., et al.** 1999. Adenovirus infections in hematopoietic stem cell transplant recipients. Clin. Infect. Dis. **29:**1494–1501.
9. **Ison, M. G.** 2006. Adenovirus infections in transplant recipients. Clin. Infect. Dis. **43:**331–339.
10. **Leen, A. M., and C. M. Rooney.** 2005. Adenovirus as an emerging pathogen in immunocompromised patients. Br. J. Haematol. **128:**135–144.
11. **Leruez-Ville, M., et al.** 2004. Real-time blood plasma polymerase chain reaction for management of disseminated adenovirus infection. Clin. Infect. Dis. **38:**45–52.
12. **Lion, T., et al.** 2003. Molecular monitoring of adenovirus in peripheral blood after allogeneic bone marrow transplantation permits early diagnosis of disseminated disease. Blood **102:**1114–1120.
13. **Lion, T., et al.** 2010. Monitoring of adenovirus load in stool by real-time PCR permits early detection of impending invasive infection in patients after allogeneic stem cell transplantation. Leukemia **24:**706–714.
14. **Nagasawa, H., et al.** 2006. A case of non-herpetic acute limbic encephalitis associated with a type-2 adenovirus infection. Rinsho Shinkeigaku **46:**322–327.
15. **Ohtsuki, N., S. Kimura, and A. Nezu.** 2000. Three cases with acute encephalopathy related with adenovirus type 7 infection. No To Hattatsu **32:**68–72.
16. **Quenelle, D. C., et al.** 2010. Efficacy of CMX001 against herpes simplex virus infections in mice and correlations with drug distribution studies. J. Infect. Dis. **202:**1492–1499.
17. **Robin, M., et al.** 2007. Disseminated adenovirus infections after allogeneic hematopoietic stem cell transplantation: incidence, risk factors and outcome. Haematologica **92:**1254–1257.
18. **Runde, V., et al.** 2001. Adenoviral infection after allogeneic stem cell transplantation (SCT): report on 130 patients from a single SCT unit involved in a prospective multi center surveillance study. Bone Marrow Transplant. **28:**51–57.
19. **Schnurr, D., A. Bollen, L. Crawford-Miksza, M. E. Dondero, and S. Yagi.** 1995. Adenovirus mixture isolated from the brain of an AIDS patient with encephalitis. J. Med. Virol. **47:**168–171.
20. **van Tol, M. J., et al.** 2005. Adenovirus infection in children after allogeneic stem cell transplantation: diagnosis, treatment and immunity. Bone Marrow Transplant. **35**(Suppl. 1):S73–S76.
21. **Vincentelli, C., M. J. Schniederjan, and D. J. Brat.** 2010. 35-year-old HIV-positive woman with basal forebrain mass. Brain Pathol. **20:**265–268.
22. **Walls, T., A. G. Shankar, and D. Shingadia.** 2003. Adenovirus: an increasingly important pathogen in paediatric bone marrow transplant patients. Lancet Infect. Dis. **3:**79–86.
23. **Zagardo, M. T., C. B. Shanholtz, G. H. Zoarski, and M. I. Rothman.** 1998. Rhombencephalitis caused by adenovirus: MR imaging appearance. AJNR Am. J. Neuroradiol. **19:**1901–1903.